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Bernstein Center for Computational Neuroscience, Humboldt University of Berlin, Philippstr. 13 Haus 6, 10115 Berlin, Germany.
E-mail: michael.brecht@bccn-berlin.de

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Cell Polarity: Stretching Prevents Developmental Cramps

Initiation and successive development of organs induce mechanical stresses at the cellular level. Using the tomato shoot apex, a new study now proposes that mechanical strain regulates the plasma membrane abundance of the PIN1 auxin transporter, thereby reinforcing a positive feed-back loop between growth and auxin accumulation.

Hongjiang Li, Jiří Friml, and Wim Grunewald

Coordinated cell and tissue polarization is crucial during both plant and animal development and requires an elaborate control system with multiple feed-back mechanisms. In plants, the polar localization of the PIN auxin transporters is pivotal for the directional transport of the signaling molecule auxin [1]. This transport is responsible for the generation of auxin gradients, which then trigger specific molecular programs to regulate organogenesis in response to developmental and environmental cues [2]. Auxin itself feeds back on tissue and organ polarity, through transcriptional and post-translational mechanisms regulating PIN localization [3,4]. Besides this physiological control, plant morphogenesis is also regulated by the mechanical properties of individual cells. Microtubules, dynamic components of the cytoskeleton, form an ordered cortical array within the cell. In growing plant cells, this array is typically oriented transverse to the growth direction, allowing growth in one direction while restricting it in other directions [5]. An extra level of mechanical constraint is exerted by the cell wall. Unlike animals, plant cells are encaged by cell walls, which they share with their neighbors. Interestingly, the presence of these neighboring cells has been shown to affect microtubule organization [6]. Growth and division of cells that are glued to each other thus induce considerable mechanical stresses on both the cellular and tissue level.

Recently, it was shown in the *Arabidopsis* shoot apical meristem that microtubules reorient upon mechanical stress [7]. This provided a paradigm in which mechanical signals, triggered by the growth of an organ, feedback on microtubule orientation and thus morphogenesis. Moreover, it was found that the orientation of subcortical microtubule arrays is highly correlated with PIN1 polarity [8], and computer models predicted that PIN1 proteins would preferentially localize to plasma membrane regions with the highest mechanical strain [8]. However, experimental evidence for the impact of mechanical stress on PIN-mediated auxin transport was so far missing.

Using osmotic treatments, external force applications, membrane modulations and growth induction, Nakayama and colleagues [9] report in this issue of *Current Biology* that growth-induced mechanical strain upregulates PIN1 function and auxin accumulation in the tomato shoot apex. These findings thus add another layer of feedback on coordinated plant growth and development, i.e. growth-induced mechanical stresses that promote auxin-mediated growth.

At the plasma membrane region with the highest mechanical tension, Nakayama *et al.* [9] observed an increased PIN1–GFP signal. However, since the established PIN1 polarity was not altered, it seems that mechanical strain affects PIN1 abundance at the predefined polar domains rather than PIN polarity *in se*. The authors hypothesize that their findings are most probably achieved by a general increase of exocytosis and reduced endocytosis. This would imply that the cellular response to mechanical stress is a universal phenomenon for all recycling plant plasma membrane proteins. Although the putative involvement of intracellular trafficking integrates the role of mechanical stress into the current understanding

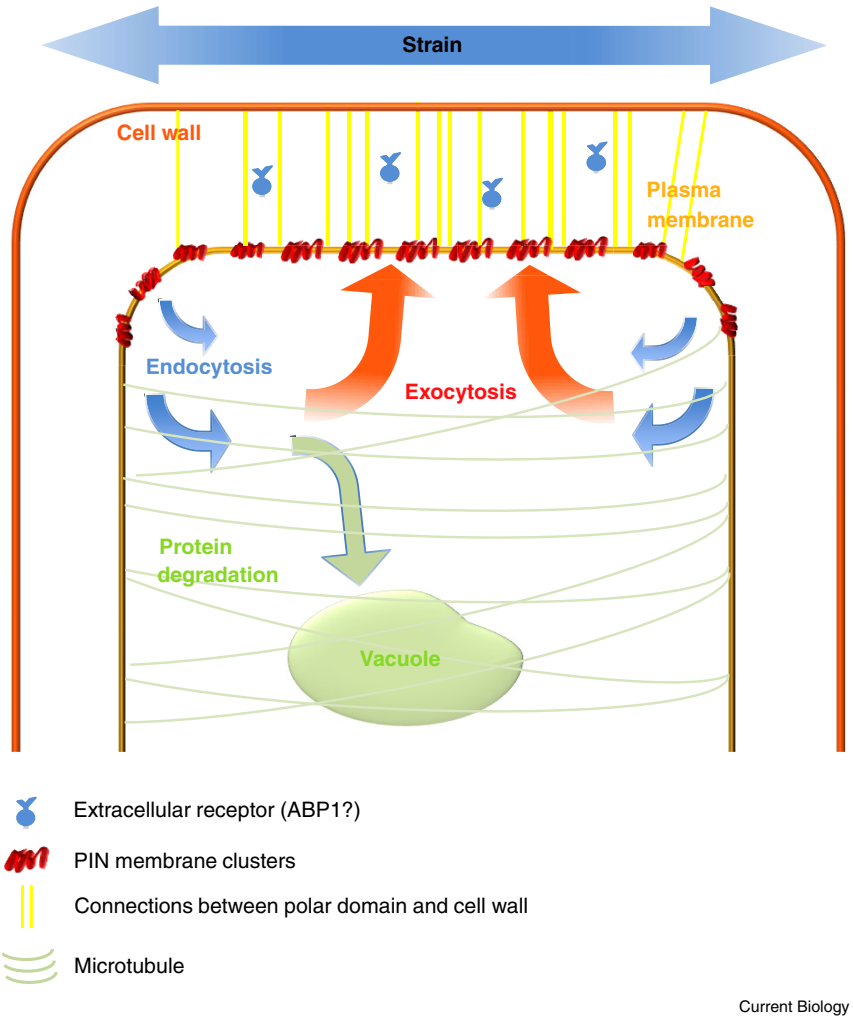

Figure 1. Putative model on how cell wall strain might feed back on PIN-mediated auxin transport.

The mechanical strain of the cell wall is probably transmitted towards the plasma membrane by so far undiscovered connections (yellow vertical stripes). The higher strain at the upper cell side increases PIN protein abundance (red membrane clusters), most probably by preferentially increased exocytosis (orange arrows) and reduced endocytosis (blue arrows). This process might be mediated by the extracellular receptor ABP1 (blue dots). At the lateral sides, a reduced strain will result in less inhibited endocytosis and accordingly PIN proteins will be more internalized. By the intracellular trafficking pathways, the PINs can recycle to those plasma membrane domains with highest mechanical strain, or can be targeted for degradation in the vacuole (green).

of polarity initiation and maintenance of polarized PIN distribution [10], it remains to be seen whether other membrane proteins (known to be as rapidly recycled as PIN proteins) undergo similar abundance changes. Additionally, uptake experiments using endocytic tracers such as FM4-64 that directly visualize endocytosis, or photo-bleaching/conversion studies of fluorescently labeled PINs to observe PIN exocytosis at mechanically stressed membranes, would be helpful in further testing this model.

In their paper, the Kuhlemeier group also shows that modulation of the membrane's properties by chemicals or temperature changes mimics the mechanical regulation of PIN1 abundance at the plasma membrane. Therefore, they propose an intriguing model in which the plasma membrane acts as a sensor for tissue mechanical stress.

Earlier studies showed a similar effect of auxin itself on PIN endocytosis [11] and might hint at a role for the extracellular Auxin Binding Protein 1 (ABP1) in the mechanical feedback on PIN polarity. Recent studies demonstrated that APB1 signaling and downstream ROP-dependent cytoskeletal rearrangements act as crucial factors in clathrin-mediated endocytosis and might thus integrate auxin signaling and PIN internalization [12–15]. By this mechanism, cells can maintain PIN auxin exporters at the plasma membrane to promote auxin efflux [11]. Theoretical models suggest that feedback on PIN internalization by extracellular receptors (such as ABP1) might indeed be sufficient to mediate cellular polarity of PIN localization and thus to (re)polarize auxin fluxes and tissues during organogenesis [5]. As the work of Nagayama *et al.* [9] here shows, the regulation of PIN trafficking by mechanics might act in parallel with auxin-mediated feedback and both processes together might provide a robust mechanism for regulating dynamic auxin distribution during organogenesis.

An important question arising from the Nakayama *et al.* [9] study is how PIN endocytic trafficking could be affected by mechanical stress. In particular, how is the signal between the plasma membrane and the cell wall transmitted? The plasma membrane might act as the sensor, yet it is the cell wall that has to deal with the mechanical impact and pressure of neighboring cells. A recent study demonstrated that the asymmetric localization of PIN proteins is maintained by connections between polar domains at the plasma membrane and the cell wall [16]. A gradual plasmolysis of *Arabidopsis* root cells revealed that before disconnection of the plasma membrane, a substantial amount of PINs remain attached to the cell wall. Together with the complete loss of asymmetric PIN1 and PIN2 localization in cell-wall lacking protoplasts [16], this clearly argues for the existence of so far unknown cell-wall-associated determinants of cell polarity. These components could transmit the mechanical tension and thus mediate the cell wall regulation of plasma membrane protein dynamics. In line with this, it could be shown that PIN proteins localize to membrane clusters and that these clusters reduce the lateral diffusion of PINs within the plasma membrane [17]. A fascinating possibility for which experimental data are lacking so far is that ABP1 might integrate both the auxin signal

and the mechanical strain of the plasma membrane and cell wall (Figure 1).

Undoubtedly, this recent work of Nakayama *et al.* [9] will influence our future models and views on developmental and environmental control of auxin-mediated growth. Previously published reports of mechanical regulation of development, for example, lateral root formation after mechanical bending of *Arabidopsis* roots [18,19], can now be better explained by these new insights. Moreover, although the auxin- and PIN-mediated polarity generation system is absent in animals, mechanical stress in animal cells is also known to trigger changes in the cytoskeleton [20]. It is thus possible that the proposed differential exo- and endocytosis of polarity components in response to membrane tension is a widespread phenomenon.

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Department of Plant Systems Biology, VIB, Technologiepark 927, B-9052 Gent, Belgium and Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 927, B-9052 Gent, Belgium.
E-mail: jifri@psb.vib-ugent.be

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Color Vision: Retinal Blues

Two complementary studies have resolved the circuitry underlying green–blue color discrimination in the retina. A blue-sensitive interneuron provides the inhibitory signal required for computing green–blue color opponency.

Jamie Johnston, Federico Esposti, and Leon Lagnado

Our ability to detect different colors relies on color-sensitive receptors in the retina, named cones. In primates there are three types of cone, sensitive to either blue, green or red, but in most other mammals there are only two types, blue and green. Despite a limited number of cone types, we are able to detect a myriad colors; this is achieved by comparing the response from different cones. For example, the response from blue cones is compared with that from green cones to give colors along the blue-green axis.

The cells in the retina performing such comparisons are called color opponent ganglion cells. Ganglion cells that compare blue and green light can be classed as blue-ON/green-OFF, excited by blue but inhibited by green light, or they can be classed as green-ON/blue-OFF, excited by green but inhibited by blue. To understand the neural circuits by which these color-opponent ganglion cells are built it is important to realize that cones do not send visual signals to ganglion cells directly, but through a class of relay neuron called bipolar cells (Figure 1A). Depending on the type of cone that they receive inputs from, bipolar cells are tuned to be most sensitive either to blue or green light. Crucially, bipolar cells also fall into two classes distinguished by the polarity of their response to an increase in light intensity. Hence, in the retina one can find both green-ON and green-OFF bipolar cells, excited by increments or decrements in the intensity of green light.

There is good evidence that, in primates, a blue-ON/green-OFF ganglion cell is built by pooling inputs from both blue-ON and green-OFF bipolar cells [1–3]; this canonical